

Summary of Sequences of Representative luciferases and the reductase set forth in the Sequence Listing

1. SEQ ID NO. 1 *Renilla reinformis* Luciferase [U.S. Patent No. 5,418,155]
- 5 2. SEQ ID NO. 2 *Cypridina hilgendorffii* luciferase [EP 0 387 355]
3. SEQ ID NO. 3 Modified *Luciola cruciata* Luciferase [firefly; U.S. Patent No. 4,968,613]
4. SEQ ID NO. 4 *Vargula (Cypridina)* luciferase [Thompson et al. (1989) Proc. Natl. Acad. Sci. U.S.A. 86:6567-6571 and from JP 3-
- 10 30678 Osaka]
5. SEQ ID NO. 5 Apoaequorin-encoding gene [U S. Patent No. 5,093,240, pAQ440]
6. SEQ ID NO. 6 Recombinant *Aequorin* AEQ1 [Prasher et al. (1987) "Sequence Comparisons of cDNAs Encoding for Aequorin
- 15 Isotypes," Biochemistry 26:1326-1332]
7. SEQ ID NO. 7 Recombinant *Aequorin* AEQ2 [Prasher et al. (1987)]
8. SEQ ID NO. 8 Recombinant *Aequorin* AEQ3 [Prasher et al. (1987)]
9. SEQ ID NO. 9 *Aequorin* photoprotein [Charbonneau et al. (1985) "Amino Acid Sequence of the Calcium-Dependent Photoprotein
- 20 Aequorin," Biochemistry 24:6762-6771]
10. SEQ ID NO. 10 *Aequorin* mutant with increased bioluminescence activity [U.S. Patent No. 5,360,728; Asp 124 changed to Ser]
11. SEQ ID NO. 11 *Aequorin* mutant with increased bioluminescence activity [U.S. Patent No. 5,360,728; Glu 135 changed to Ser]
- 25 12. SEQ ID NO. 12 *Aequorin* mutant with increased bioluminescence activity [U.S. Patent No. 5,360,728 Gly 129 changed to Ala]
13. SEQ ID NO. 13 Recombinant apoaequorin [sold by Sealite, Sciences, Bogart, GA as AQUALITE®, when reconstituted to form aequorin]
- 30 14. SEQ ID NO. 14 *Vibrio fischeri* Flavin reductase [U.S. Patent No. 5,484,723]

SEQUENCE LISTING

- (1) GENERAL INFORMATION
- (i) APPLICANT: Bryan, Bruce
- (ii) TITLE OF INVENTION: BIOLUMINESCENT ARTICLES OF MANUFACTURE
- (iii) NUMBER OF SEQUENCES: 14
- (iv) CORRESPONDENCE ADDRESS:
- (A) ADDRESSEE: Heller Ehrman White & McAuliffe
- (B) STREET: 4250 Executive Square, 7th Floor
- (C) CITY: La Jolla
- (D) STATE: CA
- (E) COUNTRY: USA
- (F) ZIP: 92037
- (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Diskette
- (B) COMPUTER: IBM Compatible
- (C) OPERATING SYSTEM: DOS
- (D) SOFTWARE: FastSEQ Version 1.5
- (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER: 09/444,762
- (B) FILING DATE:
- (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
- (A) APPLICATION NUMBER: 09/444,762
- (B) FILING DATE: 11-22-99
- (C) CLASSIFICATION:
- (viii) PRIOR APPLICATION DATA:
- (A) APPLICATION NUMBER: 08/757,046
- (B) FILING DATE: 11-25-96
- (C) CLASSIFICATION:
- (viii) PRIOR APPLICATION DATA:
- (A) APPLICATION NUMBER: 08/597,274
- (B) FILING DATE: 02-06-96
- (ix) TELECOMMUNICATION INFORMATION:
- (A) TELEPHONE: 619-450-8400
- (B) TELEFAX: 619-450-8499
- (C) TELEX:
- (2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1196 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
 (B) LOCATION: 1...942
 (D) OTHER INFORMATION: Renilla Reinformis Luciferase

(x) PUBLICATION INFORMATION:

PATENT NO.: 5,418,155

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AGC TTA AAG ATG ACT TCG AAA GTT TAT GAT CCA GAA CAA AGG AAA CGG	48
Ser Leu Lys Met Thr Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg	
1 5 10 15	
ATG ATA ACT GGT CCG CAG TGG TGG GCC AGA TGT AAA CAA ATG AAT GTT	96
Met Ile Thr Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val	
20 25 30	
CTT GAT TCA TTT ATT AAT TAT TAT GAT TCA GAA AAA CAT GCA GAA AAT	144
Leu Asp Ser Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn	
35 40 45	
GCT GTT ATT TTT TTA CAT GGT AAC GCG GCC TCT TCT TAT TTA TGG CGA	192
Ala Val Ile Phe Leu His Gly Asn Ala Ala Ser Ser Tyr Leu Trp Arg	
50 55 60	
CAT GTT GTG CCA CAT ATT GAG CCA GTA GCG CGG TGT ATT ATA CCA GAT	240
His Val Val Pro His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp	
65 70 75 80	
CTT ATT GGT ATG GGC AAA TCA GGC AAA TCT GGT AAT GGT TCT TAT AGG	288
Leu Ile Gly Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg	
85 90 95	
TTA CTT GAT CAT TAC AAA TAT CTT ACT GCA TGG TTG AAC TTC TTA ATT	336
Leu Leu Asp His Tyr Lys Tyr Leu Thr Ala Trp Leu Asn Phe Leu Ile	
100 105 110	
TAC CAA AGA AGA TCA TTT TTT GTC GGC CAT GAT TGG GGT GCT TGT TTG	384
Tyr Gln Arg Arg Ser Phe Phe Val Gly His Asp Trp Gly Ala Cys Leu	
115 120 125	
GCA TTT CAT TAT AGC TAT GAG CAT CAA GAT ARG ATC AAA GCA ATA GTT	432
Ala Phe His Tyr Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val	
130 135 140	
CAC GCT GAA AGT GTA GTA GAT GTG ATT GAA TCA TGG GAT GAA TGG CCT	480
His Ala Glu Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro	
145 150 155 160	
GAT ATT GAA GAA GAT ATT GCG TTG ATC AAA TCT GAA GAA GGA GAA AAA	528
Asp Ile Glu Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys	
165 170 175	
ATG GTT TTG GAG AAT AAC TTC TTC GTG GAA ACC ATG TTG CCA TCA AAA	576
Met Val Leu Glu Asn Asn Phe Phe Val Glu Thr Met Leu Pro Ser Lys	
180 185 190	
ATC ATG AGA AAG TTA GAA CCA GAA GAA TTT GCA GCA TAT CTT GAA CCA	624
Ile Met Arg Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr Leu Glu Pro	
195 200 205	
TTC AAA GAG AAA GGT GAA GTT CGT CGT CCA ACA TTA TCA TGG CCT CGT	672

Phe	Lys	Glu	Lys	Gly	Glu	Val	Arg	Arg	Pro	Thr	Leu	Ser	Trp	Pro	Arg		
210						215					220						
GAA	ATC	CCG	TTA	GTA	AAA	GGT	GGT	AAA	CCT	GAC	GTT	GTA	CAA	ATT	GTT	720	
Glu	Ile	Pro	Leu	Val	Lys	Gly	Gly	Lys	Pro	Asp	Val	Val	Gln	Ile	Val		
225				230						235				240			
AGG	AAT	TAT	AAT	GCT	TAT	CTA	CGT	GCA	AGT	GAT	GAT	TTA	CCA	AAA	ATG	768	
Arg	Asn	Tyr	Asn	Ala	Tyr	Leu	Arg	Ala	Ser	Asp	Asp	Leu	Pro	Lys	Met		
				245					250					255			
TTT	ATT	GAA	TCG	GAT	CCA	GGA	TTC	TTT	TCC	AAT	GCT	ATT	GTT	GAA	GGC	816	
Phe	Ile	Glu	Ser	Asp	Pro	Gly	Phe	Phe	Ser	Asn	Ala	Ile	Val	Glu	Gly		
			260					265					270				
GCC	AAG	AAG	TTT	CCT	AAT	ACT	GAA	TTT	GTC	AAA	GTA	AAA	GGT	CTT	CAT	864	
Ala	Lys	Lys	Phe	Pro	Asn	Thr	Glu	Phe	Val	Lys	Val	Lys	Gly	Leu	His		
		275					280					285					
TTT	TCG	CAA	GAA	GAT	GCA	CCT	GAT	GAA	ATG	GGA	AAA	TAT	ATC	AAA	TCG	912	
Phe	Ser	Gln	Glu	Asp	Ala	Pro	Asp	Glu	Met	Gly	Lys	Tyr	Ile	Lys	Ser		
		290				295					300						
TTC	GTT	GAG	CGA	GTT	CTC	AAA	AAT	GAA	CAA	TAA	TTACTTTGGF	TTTTTATTTA				965	
Phe	Val	Glu	Arg	Val	Leu	Lys	Asn	Glu	Gln								
305				310													
CATTTTCCC	GGGTTTAATA	ATATAAATGT	CATTTTCAAC	AATTTTATT	TAAGTGAATA	1025											
TTTCACAGG	AACATTCATA	TATGTTGATT	AATTTAGCTC	GAAGTTTACT	CTGTCATATC	1085											
ATTTTGAAT	ATTACCTCTT	TCAATGAAAC	TTTATAAACA	GTGGTTCAAT	TAATTAATAT	1145											
ATATTATAAT	TACATTGTGT	ATGTAATAAA	CTCGGTTTTA	TTATAAAAAA	A	1196											

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1822 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 1...1665
- (D) OTHER INFORMATION: Cypridina hilgendorffii luciferase

(x) PUBLICATION INFORMATION:

PATENT NO.: EP 0 387 355 TORAY

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATG	AAG	CTA	ATA	ATT	CTG	TCT	ATT	ATA	TTG	GCC	TAC	TGT	GTC	ACA	GTC	48	
Met	Lys	Leu	Ile	Ile	Leu	Ser	Ile	Ile	Leu	Ala	Tyr	Cys	Val	Thr	Val		
1				5					10					15			
AAC	TGC	CAG	GAT	GCA	TGT	CCT	GTA	GAA	GCT	GAA	GCA	CCG	TCA	AGT	ACA	96	
Asn	Cys	Gln	Asp	Ala	Cys	Pro	Val	Glu	Ala	Glu	Ala	Pro	Ser	Ser	Thr		
			20					25				30					
CCA	ACA	GTC	CCA	ACA	TCT	TGT	GAA	GCT	AAA	GAA	GGA	GAA	TGT	ATC	GAT	144	
Pro	Thr	Val	Pro	Thr	Ser	Cys	Glu	Ala	Lys	Glu	Gly	Glu	Cys	Ile	Asp		
		35				40						45					

ACC AGA TGC GCA ACA TGT AAA CGA GAC ATA CTA TCA GAC GGA CTG TGT Thr Arg 50 Cys Ala Thr Cys 55 Lys Arg Asp Ile Leu 60 Ser Asp Gly Leu Cys	192
GAA AAT AAA CCA GGG AAG ACA TGC TGT AGA ATG TGC CAG TAT GTA ATT Glu Asn 65 Lys Pro Gly 70 Lys Thr Cys Cys Arg 75 Met Cys Gln Tyr Val Ile 80	240
GAA TCC AGA GTA GAA GCT GCT GGA TAT TTT AGA ACG TTT TAC GCC AAA Glu Ser Arg Val 85 Glu Ala Ala Gly Tyr 90 Phe Arg Thr Phe Tyr 95 Ala Lys	288
AGA TTT AAT TTT CAG GAA CCT GGT AAA TAT GTG CTG GCT CGA GGA ACC Arg Phe Asn Phe 100 Gln Glu Pro Gly Lys 105 Tyr Val Leu Ala Arg 110 Gly Thr	336
AAG GGT GGC GAC TGG TCT GTA ACC CTC ACC ATG GAG AAT CTA GAT GGA Lys Gly 115 Asp Trp Ser Val Thr 120 Leu Thr Met Glu 125 Asn Leu Asp Gly	384
CAG AAG GGA GCT GTA CTG ACT AAG ACA ACA CTG GAG GTA GTA GGA GAC Gln Lys 130 Gly Ala Val Leu Thr Lys 135 Thr Thr Leu 140 Val Val Gly Asp	432
GTA ATA GAC ATT ACT CAA GCT ACT GCA GAT CCT ATC ACA GTT AAC GGA Val Ile Asp Ile Thr 145 Gln Ala Thr Ala Asp 155 Ile Thr Val Asn Gly 160	480
GGA GCT GAC CCA GTT ATC GCT AAC CCG TTC ACA ATT GGT GAG GTG ACC Gly Ala Asp Pro 165 Val Ile Ala Asn Pro 170 Phe Thr Ile Gly 175 Glu Val Thr	528
ATT GCT GTT GTC GAA ATA CCC GGC TTC AAT ATT ACA GTC ATC GAA TTC Ile Ala Val Val Glu Ile Pro Gly Phe 185 Asn Ile Thr Val 190 Ile Glu Phe	576
TTT AAA CTA ATC GTG ATA GAT ATT CTG GGA GGA AGA TCT GTG AGA ATT Phe Lys 195 Leu Ile Val Ile Asp 200 Ile Leu Gly Gly Arg 205 Ser Val Arg Ile	624
GCT CCA GAC ACA GCA AAC AAA GGA CTG ATA TCT GGT ATC TGT GGT AAT Ala Pro 210 Asp Thr Ala Asn Lys 215 Gly Leu Ile Ser Gly Ile Cys Gly Asn	672
CTG GAG ATG AAT GAC GCT GAT GAC TTT ACT ACA GAC GCA GAT CAG CTG Leu Glu 225 Met Asn Asp Ala Asp 230 Asp Phe Thr Thr 235 Asp Ala Asp Gln Leu 240	720
GCG ATC CAA CCC AAC ATA AAC AAA GAG TTC GAC GGC TGC CCA TTC TAC Ala Ile Gln Pro Asn 245 Ile Asn Lys Glu Phe 250 Asp Gly Cys Pro Phe Tyr 255	768
GGG AAT CCT TCT GAT ATC GAA TAC TGC AAA GGT CTC ATG GAG CCA TAC Gly Asn Pro 260 Ser Asp Ile Glu Tyr Cys 265 Lys Gly Leu Met Glu Pro Tyr 270	816
AGA GCT GTA TGT CGT AAC AAT ATC AAC TTC TAC TAT TAC ACT CTG TCC Arg Ala Val Cys Arg Asn Asn 280 Ile Asn Phe Tyr Tyr 285 Thr Thr Leu Ser	864
TGC GCC TTC GCT TAC TGT ATG GGA GGA GAA GAA AGA GCT AAA CAC GTC Cys Ala Phe Ala Tyr Cys 290 Met Gly Gly Glu 300 Arg Ala Lys His Val	912
CTT TTC GAC TAT GTT GAG ACA TGC GCT GCA CCG GAA ACG AGA GGA ACG	960

Leu Phe Asp Tyr Val Glu Thr Cys Ala Ala Pro Glu Thr Arg Gly Thr	
305 310 315 320	
TGT GTT TTA TCA GGA CAT ACT TTC TAT GAC ACA TTC GAC AAA GCC AGA	1008
Cys Val Leu Ser Gly His Thr Phe Tyr Asp Thr Phe Asp Lys Ala Arg	
325 330 335	
TAT CAA TTC CAG GGC CCA TGC AAA GAG CTT CTG ATG GCC GCA GAC TGT	1056
Tyr Gln Phe Gln Gly Pro Cys Lys Glu Leu Leu Met Ala Ala Asp Cys	
340 345 350	
TAC TGG AAC ACA TGG GAT GTA AAG GTT TCA CAT AGA GAT GTT GAG TCA	1104
Tyr Trp Asn Thr Trp Asp Val Lys Val Ser His Arg Asp Val Glu Ser	
355 360 365	
TAC ACT GAG GTA GAG AAA GTA ACA ATC AGG AAA CAG TCA ACT GTA GTA	1152
Tyr Thr Glu Val Glu Lys Val Thr Ile Arg Lys Gln Ser Thr Val Val	
370 375	
GAT TTG ATT GTG GAT GGC AAG CAG GTC AAG GTT GGA GGA GTG GAT GTA	1200
Asp Leu Ile Val Asp Gly Lys Gln Val Lys Val Gly Gly Val Asp Val	
385 390 395 400	
TCT ATC CCG TAC AGT TCT GAG AAC ACA TCC ATA TAC TGG CAG GAT GGA	1248
Ser Ile Pro Tyr Ser Ser Glu Asn Thr Ser Ile Tyr Trp Gln Asp Gly	
405 410 415	
GAC ATC CTG ACG ACG GCC ATC CTA CCT GAA GCT CTT GTC GTT AAG TTC	1296
Asp Ile Leu Thr Thr Ala Ile Leu Pro Glu Ala Leu Val Val Lys Phe	
420 425 430	
AAC TTT AAG CAG CTC CTT GTA GTT CAT ATC AGA GAT CCA TTC GAT GGA	1344
Asn Phe Lys Gln Leu Leu Val Val His Ile Arg Asp Pro Phe Asp Gly	
435 440 445	
AAG ACA TGC GGC ATA TGT GGT AAC TAT AAT CAA GAT TCA ACT GAT GAT	1392
Lys Thr Cys Gly Ile Cys Gly Asn Tyr Asn Gln Asp Ser Thr Asp Asp	
450 455 460	
TTC TTT GAC GCA GAA GGA GCA TGC GCT CTG ACC CCC AAT CCC CCA GGA	1440
Phe Phe Asp Ala Glu Gly Ala Cys Ala Leu Thr Pro Asn Pro Pro Gly	
465 470 475 480	
TGT ACA GAG GAG CAG AAA CCA GAA GCT GAG CGA CTC TGC AAT AGT CTA	1488
Cys Thr Glu Glu Gln Lys Pro Glu Ala Glu Arg Leu Cys Asn Ser Leu	
485 490 495	
TTT GAT AGT TCT ATC GAC GAG AAA TGT AAT GTC TGC TAC AAG CCT GAC	1536
Phe Asp Ser Ser Ile Asp Glu Lys Cys Asn Val Cys Tyr Lys Pro Asp	
500 505 510	
CGT ATT GCA CGA TGT ATG TAC GAG TAT TGC CTG AGG GGA CAG CAA GGA	1584
Arg Ile Ala Arg Cys Met Tyr Glu Tyr Cys Leu Arg Gly Gln Gln Gly	
515 520 525	
TTC TGT GAC CAT GCT TGG GAG TTC AAA AAA GAA TGC TAC ATA AAG CAT	1632
Phe Cys Asp His Ala Trp Phe Lys Lys Glu Cys Tyr Ile Lys His	
530 535 540	
GGA GAC ACT CTA GAA GTA CCA CCT GAA TGC CAA TAA ATGAACAAAG	1678
Gly Asp Thr Leu Glu Val Pro Pro Glu Cys Gln	
545 550 555	
ATACAGAAGC TAAGACTACT ACAGCAGAAG ATAAAAGAGA AGCTGTAGTT CTTCAAAAAC	1738
AGTATATTTT GATGTACTCA TTGTTTACTT ACATAAAAAAT AAATTGTTAT TATCATRAACG	1798

TAAAGAAAAA AAAAAAAAAA AAAA

1822

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1644 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
 (B) LOCATION: 1...1644
 (D) OTHER INFORMATION: Luciola Cruciata Luciferase (Firefly)

(x) PUBLICATION INFORMATION:

PATENT NO.: 4,968,613

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG GAA AAC ATG GAA AAC GAT GAA AAT ATT GTA GTT GGA CCT AAA CCG	48
Met Glu Asn Met Glu Asn Asp Glu Asn Ile Val Val Gly Pro Lys Pro	
1 5 10 15	
TTT TAC CCT ATC GAA GAG GGA TCT GCT GGA ACA CAA TTA CGC AAA TAC	96
Phe Tyr Pro Ile Glu Glu Gly Ser Ala Gly Thr Gln Leu Arg Lys Tyr	
20 25 30	
ATG GAG CGA TAT GCA AAA CTT GGC GCA ATT GCT TTT ACA AAT GCA GTT	144
Met Glu Arg Tyr Ala Lys Leu Gly Ala Ile Ala Phe Thr Asn Ala Val	
35 40 45	
ACT GGT GTT GAT TAT TCT TAC GCC GAA TAC TTG GAG AAA TCA TGT TGT	192
Thr Gly Val Asp Tyr Ser Tyr Ala Glu Tyr Leu Glu Lys Ser Cys Cys	
50 55 60	
CTA GGA AAA GCT TTG CAA AAT TAT GGT TTG GTT GTT GAT GGC AGA ATT	240
Leu Gly Lys Ala Leu Gln Asn Tyr Gly Leu Val Val Asp Gly Arg Ile	
65 70 75 80	
CGC TTA TGC AGT GAA AAC TGT GAA GAA TTT ATT CCT GAA AIA GCC	288
Ala Leu Cys Ser Glu Asn Cys Glu Glu Phe Phe Ile Pro Val Ile Ala	
85 90 95	
GGA CTG TTT ATA GGT GTA GGT GTT GCA CCC ACT AAT GAG ATT TAC ACT	336
Gly Leu Phe Ile Gly Val Gly Val Ala Pro Thr Asn Glu Ile Tyr Thr	
100 105 110	
TTA CGT GAA CTG GTT CAC AGT TTA GGT ATC TCT AAA CCA ACA ATT GTA	384
Leu Arg Glu Leu Val His Ser Leu Gly Ile Ser Lys Pro Thr Ile Val	
115 120 125	
TTT AGT TCT AAA AAA GGC TTA GAT AAA GTT ATA ACA GTA CAG AAA ACA	432
Phe Ser Ser Lys Lys Gly Leu Asp Lys Val Ile Thr Val Gln Lys Thr	
130 135 140	
GTA ACT ACT ATT AAA ACC ATT GTT ATA CTA GAT AGC AAA GTT GAT TAT	480
Val Thr Thr Ile Lys Thr Ile Val Ile Leu Asp Ser Lys Val Asp Tyr	
145 150 155 160	
CGA GGA TAT CAA TGT CTG GAC ACC TTT ATA AAA AGA AAC ACT CCA CCA	528

Arg Gly Tyr Gln Cys Leu Asp Thr Phe Ile Lys Arg Asn Thr Pro Pro	165	170	175	
GGT TTT CAA GCA TCC AGT TTC AAA ACT GTG GAA GTT GAC CGT AAA GAA				576
Gly Phe Gln Ala Ser Ser Phe Lys Thr Val Glu Val Asp Arg Lys Glu	180	185	190	
CAA GTT GCT CTT ATA ATG AAC TCT TCG GGT TCT ACC GGT TTT CCA AAA				624
Gln Val Ala Leu Ile Met Asn Ser Ser Gly Ser Thr Gly Leu Pro Lys	195	200	205	
GGC GTA CAA CIT ACT CAC GAA AAT ACA GTC ACT AGA TTT TCT CAT GCT				672
Gly Val Gln Leu Thr His Glu Asn Thr Val Thr Arg Phe Ser His Ala	210	215	220	
AGA GAT CCG ATT TAT GGT AAC CAA GTT TCA CCA GGC ACC GCT GTT TTA				720
Arg Asp Pro Ile Tyr Gly Asn Gln Val Ser Pro Gly Thr Ala Val Leu	225	230	235	
ACT GTC GTT CCA TTC CAT CAT GGT TTT GGT ATG TTC ACT ACT CTA GGG				768
Thr Val Val Pro Phe His His Gly Phe Gly Met Phe Thr Thr Leu Gly	245	250	255	
TAT TTA ATT TGT GGT TTT CGT GTT GTA ATG TTA ACA AAA TTC GAT GAA				816
Tyr Leu Ile Cys Gly Phe Arg Val Val Met Leu Thr Lys Phe Asp Glu	260	265	270	
GAA ACA TTT TTA AAA ACT CTA CAA GAT TAT AAA TGT ACA AGT GTT ATT				864
Glu Thr Phe Leu Lys Thr Leu Gln Asp Tyr Lys Cys Thr Ser Val Ile	275	280	285	
CTT GTA CCG ACC TTG TTT GCA ATT CTC AAC AAA AGT GAA TTA CTC AAT				912
Leu Val Pro Thr Leu Phe Ala Ile Leu Asn Lys Ser Glu Leu Leu Asn	290	295	300	
AAA TAC GAT TTG TCA AAT TTA GTT GAG ATT GCA TCT GGC GGA GCA CCT				960
Lys Tyr Asp Leu Ser Asn Leu Val Glu Ile Ala Ser Gly Gly Ala Pro	305	310	315	
TTA TCA AAA GAA GTT GGT GAA GCT GTT GCT AGA CGC TTT AAT CTT CCC				1008
Leu Ser Lys Glu Val Gly Glu Ala Val Ala Arg Arg Phe Asn Leu Pro	325	330	335	
GGT GTT CGT CAA GGT TAT GGT TTA ACA GAA ACA ACA TCT GCC ATT ATT				1056
Gly Val Arg Gln Gly Tyr Gly Leu Thr Glu Thr Thr Ser Ala Ile Ile	340	345	350	
ATT ACA CCA GAA GGA GAC GAT AAA CCA GGA GCT TCT GGA AAA GTC GTG				1104
Ile Thr Pro Glu Gly Asp Asp Lys Pro Gly Ala Ser Gly Lys Val Val	355	360	365	
CCG TTG TTT AAA GCA AAA GTT ATT GAT CTT GAT ACC AAA AAA TCT TTA				1152
Pro Leu Phe Lys Ala Lys Val Ile Asp Leu Asp Thr Lys Lys Ser Leu	370	375	380	
GGT CCT AAC AGA CGT GGA GAA GTT TGT GTT AAA GGA CCT ATG CTT ATG				1200
Gly Pro Asn Arg Arg Gly Glu Val Cys Val Lys Gly Pro Met Leu Met	385	390	395	
AAA GGT TAT GTA AAT AAT CCA GAA GCA ACA AAA GAA CTT ATT GAC GAA				1248
Lys Gly Tyr Val Asn Asn Pro Glu Ala Thr Lys Glu Leu Ile Asp Glu	405	410	415	
GAA GGT TGG CTG CAC ACC GGA GAT ATT GGA TAT TAT GAT GAA GAA AAA				1296
Glu Gly Trp Leu His Thr Gly Asp Ile Gly Tyr Tyr Asp Glu Glu Lys				

420										425										430										
CAT	TTC	TTT	ATT	GTC	GAT	CGT	TTG	AAG	TCT	TTA	ATC	AAA	TAC	AAA	GGA	1344														
His	Phe	Phe	Ile	Val	Asp	Arg	Leu	Lys	Ser	Leu	Ile	Lys	Tyr	Lys	Gly															
		435						440				445																		
TAC	CAA	GTA	CCA	CCT	GCC	GAA	TTA	GAA	TCC	GTT	CTT	TTG	CAA	CAT	CCA	1392														
Tyr	Gln	Val	Pro	Pro	Ala	Glu	Leu	Glu	Ser	Val	Leu	Leu	Gln	His	Pro															
		450				455					460																			
TCT	ATC	TTT	GAT	GCT	GGT	GTT	GCC	GGC	GTT	CCT	GAT	CCT	GTA	GCT	GGC	1440														
Ser	Ile	Phe	Asp	Ala	Gly	Val	Ala	Gly	Val	Pro	Asp	Pro	Val	Ala	Gly															
		465			470				475					480																
GAG	CTT	CCA	GGA	GCC	GTT	GTT	GTA	CTG	GAA	AGC	GGA	AAA	AAT	ATG	ACC	1488														
Glu	Leu	Pro	Gly	Ala	Val	Val	Val	Leu	Glu	Ser	Gly	Lys	Asn	Met	Thr															
				485					490					495																
GAA	AAA	GAA	GTA	ATG	GAT	TAT	GTT	GCA	AGT	CAA	GTT	TCA	AAT	GCA	AAA	1536														
Glu	Lys	Glu	Val	Met	Asp	Tyr	Val	Ala	Ser	Gln	Val	Ser	Asn	Ala	Lys															
			500					505					510																	
CGT	TTA	CGT	GGT	GGT	GTT	CGT	TTT	GTG	GAT	GAA	GTA	CCT	AAA	GGT	CTT	1584														
Arg	Leu	Arg	Gly	Gly	Val	Arg	Phe	Val	Asp	Glu	Val	Pro	Lys	Gly	Leu															
		515				520						525																		
ACT	GGA	AAA	ATT	GAC	GGC	AGA	GCA	ATT	AGA	GAA	ATC	CTT	AAG	AAA	CCA	1632														
Thr	Gly	Lys	Ile	Asp	Gly	Arg	Ala	Ile	Arg	Glu	Ile	Leu	Lys	Lys	Pro															
		530				535					540																			
GTI	GCT	AAG	ATG													1644														
	Val	Ala	Lys	Met																										
		545																												

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1820 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 1...1664
- (D) OTHER INFORMATION: Vargula (cypridina) luciferase

(x) PUBLICATION INFORMATION:

- JP 3-30678 Osaka (Tsuji)
- (A) AUTHORS: Thompson *et al.*
- (C) JOURNAL: Proc. Natl. Acad. Sci. U.S.A.
- (D) VOLUME: 86
- (F) PAGES: 1326-1332
- (G) DATE: (1989)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ATG	AAG	ATA	ATA	ATT	CTG	TCT	GTT	ATA	TTG	GCC	TAC	TGT	GTC	ACC	GAC	48
Met	Lys	Ile	Ile	Ile	Leu	Ser	Val	Ile	Leu	Ala	Tyr	Cys	Val	Thr	Asp	
1				5				10					15			
AAC	TGT	CAA	GAT	GCA	TGT	CCT	GTA	GAA	GCG	GAA	CCG	CCA	TCA	AGT	ACA	96
Asn	Cys	Gln	Asp	Ala	Cys	Pro	Val	Glu	Ala	Glu	Pro	Pro	Ser	Ser	Thr	

20										25										30										
CCA Pro	ACA Thr	GTT Val 35	CCA Pro	ACT Thr	TCT Ser	TGT Cys	GAA Glu 40	GCT Ala	AAA Lys	GAA Glu	GGA Gly	GAA Glu 45	TGT Cys	ATA Ile	GAT Asp	144														
ACC Thr	AGA Arg 50	TGC Cys	GCA Ala	ACA Thr	TGT Cys	AAA Lys 55	CGA Arg	GAT Asp	ATA Ile	CTA Leu	TCA Ser 60	GAT Asp	GGA Gly	CTG Leu	TGT Cys	192														
GAA Glu 65	AAT Asn	AAA Lys	CCA Pro	GGG Gly	AAG Lys 70	ACA Thr	TGC Cys	TGT Cys	AGA Arg	ATG Met 75	TGC Cys	CAG Gln	TAT Tyr	GTG Val 80	ATT Ile	240														
GAA Glu	TGC Cys	AGA Arg	GTA Val	GAA Glu 85	GCA Ala	GCT Ala	GGT Gly	TAT Tyr	TTT Phe 90	AGA Arg	ACG Thr	TTT Phe	TAC Tyr	GGC Gly 95	AAA Lys	288														
AGA Arg	TTT Phe	AAT Asn 100	TTT Phe	CAG Gln	GAA Glu	CCT Pro	GGT Gly	AAA Lys 105	TAT Tyr	GTG Val	CTG Leu	GCT Ala	AGG Arg 110	GGA Gly	ACC Thr	336														
AAG Lys	GGT Gly	GGC Gly 115	GAT Asp	TGG Trp	TCT Ser	GTA Val	ACC Thr 120	CTC Leu	ACC Thr	ATG Met	GAG Glu	AAT Asn 125	CTA Leu	GAT Asp	GGA Gly	384														
CAG Gln	AAG Lys 130	GGA Gly	GCT Ala	GTG Val	CTG Leu	ACT Thr 135	AAG Lys	ACA Thr	ACA Thr	CTG Leu	GAG Glu 140	GTT Val	GCA Ala	GGA Gly	GAC Asp	432														
GTA Val 145	ATA Ile	GAC Asp	ATT Ile	ACT Thr	CAA Gln 150	GCT Ala	ACT Thr	GCA Ala	GAT Asp	CCT Pro 155	ATC Ile	ACA Thr	GTT Val	AAC Asn	GGA Gly 160	480														
GGA Gly	GCT Ala	GAC Asp	CCA Pro 165	GTT Val	ATC Ile	GCT Ala	AAC Asn	CCG Pro	TTC Phe 170	ACA Thr	ATT Ile	GGT Gly	GAG Glu	GTG Val 175	ACC Thr	528														
ATT Ile	GCT Ala	GTT Val 180	GTT Val	GAA Glu	ATA Ile	CCG Pro	GGC Gly	TTC Phe 185	AAT Asn	ATC Thr	ACA Thr	GTC Val 190	ATC Ile	GAA Glu	TTC Phe	576														
TTT Phe	AAA Lys 195	CTA Leu	ATC Ile	GTG Val	ATT Ile	GAT Asp	ATT Ile 200	CTG Leu	GGA Gly	GGA Gly	AGA Arg	TCT Ser 205	GTC Val	AGA Arg	ATT Ile	624														
GCT Ala	CCA Pro 210	GAC Asp	ACA Thr	GCA Ala	AAC Asn	AAA Lys 215	GGA Gly	CTG Leu	ATA Ile	TCT Ser	GGT Gly 220	ATC Ile	TGT Cys	GGT Gly	AAT Asn	672														
CTG Leu 225	GAG Glu	ATG Met	AAT Asn	GAC Asp	GCT Ala 230	GAT Asp	GAC Asp	TTT Phe	ACT Thr	ACA Thr 235	GAT Asp	GCA Ala	GAT Asp	CAG Gln 240	CTG Leu 240	720														
GCG Ala	ATC Ile	CAA Gln	CCC Pro	AAC Asn 245	ATA Ile	AAC Asn	AAA Lys	GAG Glu 250	TTC Phe	GAC Asp 255	GGC Gly	TGC Cys	CCA Pro	TTC Phe 255	TAT Tyr	768														
GGC Gly	AAT Asn	CCT Pro	TCT Ser 260	GAT Asp	ATC Ile	GAA Glu	TAC Tyr	TGC Cys 265	AAA Lys	GGT Gly	CTG Leu	ATG Met	GAG Glu 270	CCA Pro	TAC Tyr	816														
AGA Arg	GCT Ala	GTA Val 275	TGT Cys	CGT Arg	AAC Asn	AAT Asn	ATC Ile 280	AAC Asn	TTC Phe	TAC Tyr	TAT Tyr	TAC Tyr 285	ACT Thr	CTA Leu	TCC Ser	864														

TGT Cys	GCC Ala	TTC Phe	GCT Ala	TAC Tyr	TGT Cys	ATG Met	GGA Gly	GGA Gly	GAA Glu	GAA Glu	AGA Pro	GCT Ala	AAA Lys	CAC His	GTC Val	912
290						295					300					
CTT Leu	TTC Phe	GAC Asp	TAT Tyr	GTT Val	GAG Glu	ACA Thr	TGC Cys	GCT Ala	CGC Ala	CCG Ala	GAA Glu	ACG Thr	AGA Arg	GGA Gly	ACG Thr	960
305					310					315					320	
TGT Cys	GTT Val	TTA Leu	TCA Ser	GGA Ser	CAT His	ACT Thr	TTC Phe	TAT Tyr	GAC Thr	ACA Thr	TTC Phe	GAC Asp	AAA Lys	GCA Ala	AGA Arg	1008
				325					330				335			
TAT Tyr	CAA Gln	TTC Phe	CAG Gln	GGC Gly	CCA Pro	TGC Cys	AAG Lys	GAG Ile	ATT Leu	CTG Met	ATG Ala	GCC Ala	GCA Asp	GAC Cys	TGT Cys	1056
				340				345					350			
TAC Tyr	TGG Trp	AAC Asn	ACA Thr	TGG Thr	GAT Asp	GTA Val	AAG Lys	GTT Val	TCA Ser	CAT His	AGA Arg	GAC Asp	GTC Val	GAA Glu	TCA Ser	1104
		355					360					365				
TAC Tyr	ACT Thr	GAG Glu	GTA Val	GAG Glu	AAA Lys	GTA Thr	ACA Thr	ATC Ile	AGG Arg	AAA Lys	CAG Gln	TCA Ser	ACT Thr	GTA Val	GTA Val	1152
		370				375					380					
GAT Asp	CTC Leu	ATT Ile	GTG Val	GAT Asp	GGC Gly	AAG Lys	CAG Gln	GTC Val	AAG Lys	GTT Val	GGA Gly	GAG Val	GAT Asp	GTA Val		1200
385					390				395					400		
TCT Ser	ATC Ile	CCG Pro	TAC Tyr	AGC Ser	TCT Ser	GAG Glu	AAC Asn	ACT Thr	TCC Ser	ATA Ile	TAC Tyr	TGG Trp	CAG Gln	GAT Asp	GGA Gly	1248
				405					410				415			
GAC Asp	ATC Ile	CTG Leu	ACG Thr	ACG Thr	GCC Ala	ATC Ile	CTA Leu	CCT Pro	GAA Glu	GCT Ala	CTT Val	GTC Val	GTT Val	AAG Lys	TTC Phe	1296
				420				425					430			
AAC Asn	TTT Phe	AAG Lys	CAG Gln	CTC Leu	CTT Leu	GTA Val	GTT Val	CAT His	ATC Ile	AGA Arg	GAT Asp	CCA Pro	TTC Phe	GAT Asp	GCA Ala	1344
		435					440					445				
AAG Lys	ACA Thr	TGC Cys	GGC Gly	ATA Ile	TGT Cys	GGT Cys	AAC Gly	TAT Asn	AAT Tyr	CAA Asn	GAT Gln	ACT Asp	GAT Thr	GAT Asp		1392
				450			455				460					
TTC Phe	TTT Asp	GAC Phe	GCA Ala	GAA Glu	GGA Gly	GCA Ala	TGC Cys	GCT Ala	ACC Leu	CCC Pro	AAC Asn	CCC Pro	CCA Pro	GGA Gly		1440
465					470				475				480			
TGT Cys	ACA Thr	GAG Glu	GAA Glu	CAG Gln	AAA Lys	CCA Pro	GAA Glu	GCT Ala	GAG Arg	CGA Leu	CTT Cys	TGC Asn	AAT Asn	CTC Leu		1488
				485				490					495			
TTT Phe	GAT Asp	TCT Ser	TCT Ile	ATC Asp	GAC Glu	GAG Lys	AAA Lys	TGT Cys	AAT Asn	GTC Val	TGC Cys	TAC Tyr	AAG Lys	CCT Pro	GAC Asp	1536
				500				505					510			
CGG Arg	ATT Ile	GCC Ala	CGA Arg	TGT Cys	ATG Met	TAC Tyr	GAG Glu	TAT Tyr	TGC Cys	CTG Leu	AGG Arg	GGA Gly	CAA Gln	CAA Gln	GGA Gly	1584
		515					520					525				
TTT Phe	TGT Cys	GAC Asp	CAT His	GCT Ala	TGG Trp	GAG Phe	TTC Lys	AAG Lys	AAA Lys	GAA Cys	TGC Tyr	TAC Ile	ATA Lys	AAA His	CAT His	1632
		530				535					540					
GGA Gly	GAC Asp	ACT Thr	CTA Leu	GAA Glu	GTA Val	CCA Pro	CCT Pro	GAA Glu	TGT Cys	CAA Cys	TAA Cys	ACGTACAAAG				1678

545

550

555

ATACAGAAAGC TAAGGCTACT ACAGCAGAAG ATAAAAAAGA AACTGTAGTT CCTTCAAAAA 1738
 CCGTGTATTT TATGTACTCA TTGTTTAATT AGAGCAAAAT AAATGTGTAT TATCATAACT 1798
 TAAACTAAAA AAAAAAAAAA AA 1820

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 958 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 115...702

(D) OTHER INFORMATION: apoaeguorin-encoding gene

(x) PUBLICATION INFORMATION:

PATENT NO.: 5,093,240

(A) AUTHORS: Incouye *et al.*

(C) JOURNAL: Proc. Natl. Acad. Sci. U.S.A.

(D) VOLUME: 82

(F) PAGES: 3154-3158

(G) DATE: (1985)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GGGGGGGGGG GGGGGGGGGG GGGGGGGGGG GGGGAATGCAA TTCATCTTTG CATCAAAGAA 60
 TTACATCAAA TCTCTAGTTG ATCAACTAAA TTGTCTCGAC AACACAAAGC AAAC ATG 117
 Met
 1
 ACA AGC AAA CAA TAC TCA GTC AAG CTT ACA TCA GAC TTC GAC AAC CCA 165
 Thr Ser Lys Gln Tyr Ser Val Lys Leu Thr Ser Asp Phe Asp Asn Pro
 5 10 15
 AGA TGG ATT GGA CGA CAC AAG CAT ATG TTC AAT TTC CTT GAT GTC AAC 213
 Arg Trp Ile Gly Arg His Lys His Met Phe Asn Phe Leu Asp Val Asn
 20 25 30
 CAC AAT GGA AAA ATC TCT CTT GAC GAG ATG GTC TAC AAG GCA TCT GAT 261
 His Asn Gly Lys Ile Ser Leu Asp Glu Met Val Tyr Lys Ala Ser Asp
 35 40 45
 ATT GTC ATC AAT AAC CTT GGA GCA ACA CCT GAG CAA GCC AAA CGA CAC 309
 Ile Val Ile Asn Asn Leu Gly Ala Thr Pro Glu Gln Ala Lys Arg His
 50 55 60 65
 AAA GAT GCT GTA GAA GCC TTC TTC GGA GGA GCT GGA ATG AAA TAT GGT 357
 Lys Asp Ala Val Glu Ala Phe Phe Gly Gly Ala Gly Met Lys Tyr Gly
 70 75 80
 GTG GAA ACT GAT TGG CCT GCA TAT ATT GAA GGA TGG AAA AAA TTG GCT 405
 Val Glu Thr Asp Trp Pro Ala Tyr Ile Glu Gly Trp Lys Lys Leu Ala
 85 90 95

ACT GAT GAA TTG GAG AAA TAC GCC AAA AAC GAA CCA ACG CTC ATC CGT	453
Thr Asp Glu Leu Glu Lys Tyr Ala Lys Asn Glu Pro Thr Leu Ile Arg	
100 105 110	
ATA TGG GGT GAT GCT TTG TTT GAT ATC GTT GAC AAA GAT CAA AAT GGA	501
Ile Trp Gly Asp Ala Leu Phe Asp Ile Val Asp Lys Asp Gln Asn Gly	
115 120 125	
GCC ATT ACA CTG GAT GAA TGG AAA GCA TAC ACC AAA GCT GCT GGT ATC	549
Ala Ile Thr Leu Asp Glu Trp Lys Ala Tyr Thr Lys Ala Ala Gly Ile	
130 135 140 145	
ATC CAA TCA TCA GAA GAT TGC GAG GAA ACA TTC AGA GTG TGC GAT ATT	597
Ile Gln Ser Ser Glu Asp Cys Glu Glu Thr Phe Arg Val Cys Asp Ile	
150 155 160	
GAT GAA AGT GGA CAA CTC GAT GTT GAT GAG ATG ACA AGA CAA CAT TTA	645
Asp Glu Ser Gly Gln Leu Asp Val Asp Glu Met Thr Arg Gln His Leu	
165 170 175	
GGA TTT TGG TAC ACC ATG GAT CCT GCT TGC GAA AAG CTC TAC GGT GGA	693
Gly Phe Trp Tyr Thr Met Asp Pro Ala Cys Glu Lys Leu Tyr Gly Gly	
180 185 190	
GCT GTC CCC TAAGAAGCTC TACGGTGGTG ATGCACCTTA GGAAGATGAT GTGATTTTGA	752
Ala Val Pro	
195	
ATAAAACACT GATGAATCA ATCAAAATTT TCCAAATTTT TGAACGATTT CAATCGTTTG	812
TGTTGATTTT TGTAAATTAGG AACAGATTAA ATCGAATGAT TAGTTGTTTT TTTAATCAAC	872
AGAACTTACA AATCGAAAAA GTAAAAAATA AAAAAAATAA AAAAAAATAA AAAAAAATAA	932
AAAAAATAA AAAAAAATAA AAAAAA	958

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 591 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
- (B) LOCATION: 1...588
- (D) OTHER INFORMATION: Recombinant Aequorin AEQ1

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Prasher et al.
- (B) TITLE: Sequence Comparisons of Complementary DNAs Encoding Aequorin Isoforms
- (C) JOURNAL: Biochemistry
- (D) VOLUME: 26
- (F) PAGES: 1326-1332
- (G) DATE: 1987

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATG ACC AGC GAA CAA TAC TCA GTC AAG CTT ACA CCA GAC TTC GAC AAC	48
---	----

Met Thr Ser Glu Gln Tyr Ser Val Lys Leu Thr Pro Asp Phe Asp Asn
 1 5 10 15
 CCA AAA TGG ATT GGA CGA CAC AAG CAC ATG TTT AAT TTT CTT GAT GTC 96
 Pro Lys Trp Ile Gly Arg His Lys His Met Phe Asn Phe Leu Asp Val
 20 25 30
 AAC CAC AAT GGA AGG ATC TCT CTT GAC GAG ATG GTC TAC AAG GCG TCC 144
 Asn His Asn Gly Arg Ile Ser Leu Asp Glu Met Val Tyr Lys Ala Ser
 35 40 45
 GAT ATT GTT ATA AAC AAT CTT GGA GCA ACA CCT GAA CAA GCC AAA CGT 192
 Asp Ile Val Ile Asn Asn Leu Gly Ala Thr Pro Glu Gln Ala Lys Arg
 50 55 60
 CAC AAA GAT GCT GTA GAA GCC TTC TTC GGA GGA GCT GGA ATG AAA TAT 240
 His Lys Asp Ala Val Glu Ala Phe Phe Gly Gly Ala Gly Met Lys Tyr
 65 70 75
 GGT GTA GAA ACT GAA TGG CCT GAA TAC ATC GAA GGA TGG AAA AGA CTG 288
 Gly Val Glu Thr Trp Pro Glu Tyr Ile Glu Gly Trp Lys Arg Leu
 85 90 95
 GCT TCC GAG GAA TTG AAA AGG TAT TCA AAA AAC CAA ATC ACA CTT ATT 336
 Ala Ser Glu Glu Leu Lys Arg Tyr Ser Lys Asn Gln Ile Thr Leu Ile
 100 105 110
 CGT TTA TGG GGT GAT GCA TTG TTC GAT ATC ATT GAC AAA GAC CAA AAT 384
 Arg Leu Trp Gly Asp Ala Leu Phe Asp Ile Ile Asp Lys Asp Gln Asn
 115 120 125
 GGA GCT ATT TCA CTG GAT GAA TGG AAA GCA TAC ACC AAA TCT GAT GGC 432
 Gly Ala Ile Ser Leu Asp Glu Trp Lys Ala Tyr Thr Lys Ser Asp Gly
 130 135 140
 ATC ATC CAA TCG TCA GAA GAT TGC GAG GAA ACA TTC AGA GTG TGC GAT 480
 Ile Ile Gln Ser Ser Glu Asp Cys Glu Glu Thr Phe Arg Val Cys Asp
 145 150 155 160
 ATT GAT GAA AGT GGA CAG CTC GAT GTT GAT GAG ATG ACA AGA CAA CAT 528
 Ile Asp Glu Ser Gly Gln Leu Asp Val Asp Glu Met Thr Arg Gln His
 165 170 175
 TTA GGA TTT TGG TAC ACC ATG GAT CCT GCT TGC GAA AAG CTC TAC GGT 576
 Leu Gly Phe Trp Tyr Thr Met Asp Pro Ala Cys Glu Lys Leu Tyr Gly
 180 185 190
 GGA GCT GTC CCC TAA 591
 Gly Ala Val Pro *

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 591 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(ix) FEATURE:

- (A) NAME/KEY: Coding Sequence
 (B) LOCATION: 1...588
 (D) OTHER INFORMATION: Recombinant Aequorin AEQ2

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Prasher et al.
 (B) TITLE: Sequence Comparisons of Complementary DNAs Encoding Aequorin Isoforms
 (C) JOURNAL: Biochemistry
 (D) VOLUME: 26
 (F) PAGES: 1326-1332
 (G) DATE: 1987

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATG ACC AGC GAA CAA TAC TCA GTC AAG CTT ACA TCA GAC TTC GAC AAC	48
Met Thr Ser Glu Gln Tyr Ser Val Lys Leu Thr Ser Asp Phe Asp Asn	
1 5 10 15	
CCA AGA TGG ATT GGA CGA CAC AAG CAT ATG TTC AAT TTC CTT GAT GTC	96
Pro Arg Trp Ile Gly Arg His Lys His Met Phe Asn Phe Leu Asp Val	
20 25 30	
AAC CAC AAT GGA AAA ATC TCT CTT GAC GAG ATG GTC TAC AAG GCA TCT	144
Asn His Asn Gly Lys Ile Ser Leu Asp Glu Met Val Tyr Lys Ala Ser	
35 40 45	
GAT ATT GTC ATC AAT AAC CTT GGA GCA ACA CCT GAG CAA GCC AAA CGA	192
Asp Ile Val Ile Asn Asn Leu Gly Ala Thr Pro Glu Gln Ala Lys Arg	
50 55 60	
CAC AAA GAT GCT GTA GAA GCC TTC TTC GGA GGA GCT GGA ATG AAA TAT	240
His Lys Asp Ala Val Glu Ala Phe Phe Gly Ala Gly Met Lys Tyr	
65 70 75 80	
GGT GTG GAA ACT GAT TGG CCT GCA TAT ATT GAA GGA TGG AAA AAA TTG	288
Gly Val Glu Thr Asp Trp Pro Ala Tyr Ile Glu Gly Trp Lys Lys Leu	
85 90 95	
GCT ACT GAT GAA TTG GAG AAA TAC GCC AAA AAC GAA CCA ACG CTC ATC	336
Ala Thr Asp Glu Leu Glu Lys Tyr Ala Lys Asn Glu Pro Thr Leu Ile	
100 105 110	
CGT ATA TGG GGT GAT GCT TTG TTC GAT ATC GTT GAC AAA GAT CAA AAT	384
Arg Ile Trp Gly Asp Ala Leu Phe Asp Ile Val Asp Lys Asp Gln Asn	
115 120 125	
GGA GCC ATT ACA CTG GAT GAA TGG AAA GCA TAC ACC AAA GCT GCT GGT	432
Gly Ala Ile Thr Leu Asp Glu Trp Lys Ala Tyr Thr Lys Ala Ala Gly	
130 135 140	
ATC ATC CAA TCA TCA GAA GAT TGC GAG GAA ACA TTC AGA GTG TGC GAT	480
Ile Ile Gln Ser Ser Glu Asp Cys Glu Glu Thr Phe Arg Val Cys Asp	
145 150 155 160	
ATT GAT GAA AGT GGA CAA CTC GAT GTT GAT GAG ATG ACA AGA CAA CAT	528
Ile Asp Glu Ser Gly Gln Leu Asp Val Asp Glu Met Thr Arg Gln His	
165 170 175	
TTA GGA TTT TGG TAC ACC ATG GAT CCT GCT TGC GAA AAG CTC TAC GGT	576
Leu Gly Phe Trp Tyr Thr Met Asp Pro Ala Cys Glu Lys Leu Tyr Gly	
180 185 190	

GGA GCT GTC CCC TAA
Gly Ala Val Pro *
195

591

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 591 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 1...588

(D) OTHER INFORMATION: Recombinant Aequorin AEQ3

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Prasher et al.

(B) TITLE: Sequence Comparisons of Complementary DNAs Encoding Aequorin Isoforms

(C) JOURNAL: Biochemistry

(D) VOLUME: 26

(F) PAGES: 1326-1332

(G) DATE: 1987

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATG ACC AGC GAA CAA TAC TCA GTC AAG CTT ACA TCA GAC TTC GAC AAC	48
Met Thr Ser Glu Gln Tyr Ser Val Lys Leu Thr Ser Asp Phe Asp Asn	
1 5 10 15	
CCA AGA TGG ATT GGA CGA CAC AAG CAT ATG TTC AAT TTC CTT GAT GTC	96
Pro Arg Trp Ile Gly Arg His Lys His Met Phe Asn Phe Leu Asp Val	
20 25 30	
AAC CAC AAT GGA AAA ATC TCT CTT GAC GAG ATG GTC TAC AAG GCA TCT	144
Asn His Asn Gly Lys Ile Ser Leu Asp Glu Met Val Tyr Lys Ala Ser	
35 40 45	
GAT ATT GTC ATC AAT AAC CTT GGA GCA ACA CCT GAG CAA GCC AAA CGA	192
Asp Ile Val Ile Asn Asn Leu Gly Ala Thr Pro Glu Gln Ala Lys Arg	
50 55 60	
CAC AAA GAT GCT GTA GGA GAC TTC TTC GGA GGA GCT GGA ATG AAA TAT	240
His Lys Asp Ala Val Gly Asp Phe Phe Gly Gly Ala Gly Met Lys Tyr	
65 70 75 80	
GGT GTG GAA ACT GAT TGG CCT GCA TAC ATT GAA GGA TGG AAA AAA TTG	288
Gly Val Glu Thr Asp Trp Pro Ala Tyr Ile Glu Gly Trp Lys Lys Leu	
85 90 95	
GCT ACT GAT GAA TTG GAG AAA TAC GCC AAA AAC GAA CCA ACG CTC ATC	336
Ala Thr Asp Glu Leu Glu Lys Tyr Ala Lys Asn Glu Pro Thr Leu Ile	
100 105 110	
CGT ATA TGG GGT GAT GCT TTG TTC GAT ATC GTT GAC AAA GAT CAA AAT	384
Arg Ile Trp Gly Asp Ala Leu Phe Asp Ile Val Asp Lys Asp Gln Asn	

115	120	125	
GGA GCC ATT ACA CTG GAT GAA TGG AAA GCA TAC ACC AAA GCT GCT GGT			432
Gly Ala Ile Thr Leu Asp Glu Trp Lys Ala Tyr Thr Lys Ala Ala Gly			
130	135	140	
ATC ATC CAA TCA TCA GAA GAT TGC GAG GAA ACA TTC AGA GTG TGC GAT			480
Ile Ile Gln Ser Ser Glu Asp Cys Glu Glu Thr Phe Arg Val Cys Asp			
145	150	155	160
ATT GAT GAA AAT GGA CAA CTC GAT GTT GAT GAG ATG ACA AGA CAA CAT			528
Ile Asp Glu Asn Gly Gln Leu Asp Val Asp Glu Met Thr Arg Gln His			
165	170	175	
TTA GGA TTT TGG TAC ACC ATG GAT CCT GCT TGC GAA AAG CTC TAC GGT			576
Leu Gly Phe Trp Tyr Thr Met Asp Pro Ala Cys Glu Lys Leu Tyr Gly			
180	185	190	
GGA GCT GTC CCC TAA			591
Gly Ala Val Pro *			
195			

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 567 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 1...567

(D) OTHER INFORMATION: Aequorin photoprotein

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Charbonneau et al.

(B) TITLE: Amino acid sequence of the calcium-dependent photoprotein aequorin

(C) JOURNAL: Am. Chem. Soc.

(D) VOLUME: 24

(E) ISSUE: 24

(F) PAGES: 6762-6771

(G) DATE: 1985

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTC AAG CTT ACA CCA GAC TTC GAC AAC CCA AAA TGG ATT GGA CGA CAC	48		
Val Lys Leu Thr Pro Asp Phe Asp Asn Pro Lys Trp Ile Gly Arg His			
1	5	10	15
AAG CAC ATG TTT AAT TTT CTT GAT GTC AAC CAC AAT GGA AGG ATC TCT	96		
Lys His Met Phe Asn Phe Leu Asp Val Asn His Asn Gly Arg Ile Ser			
20	25	30	
CTT GAC GAG ATG GTC TAC AAG GCG TCC GAT ATT GTT ATA AAC AAT CTT	144		
Leu Asp Glu Met Val Tyr Lys Ala Ser Asp Ile Val Ile Asn Asn Leu			

35										40										45										
GGA	GCA	ACA	CCT	GAA	CAA	GCC	AAA	CGT	CAC	AAA	GAT	GCT	GTA	GAA	GCC	192														
Gly	Ala	Thr	Pro	Glu	Gln	Ala	Lys	Arg	His	Lys	Asp	Ala	Val	Glu	Ala															
	50					55					60																			
TTC	TTC	GGA	GGA	GCT	GCA	ATG	AAA	TAT	GGT	GTA	GAA	ACT	GAA	TGG	CCT	240														
Phe	Phe	Gly	Gly	Ala	Ala	Met	Lys	Tyr	Gly	Val	Glu	Thr	Glu	Trp	Pro															
65				70					75					80																
GAA	TAC	ATC	GAA	GGA	TGG	AAA	AGA	CTG	GCT	TCC	GAG	GAA	TTG	AAA	AGG	288														
Glu	Tyr	Ile	Glu	Gly	Trp	Lys	Arg	Leu	Ala	Ser	Glu	Glu	Leu	Lys	Arg															
			85					90					95																	
TAT	TCA	AAA	AAC	CAA	ATC	ACA	CTT	ATT	CGT	TTA	TGG	GGT	GAT	GCA	TTG	336														
Tyr	Ser	Lys	Asn	Gln	Ile	Thr	Leu	Ile	Arg	Leu	Trp	Gly	Asp	Ala	Leu															
		100					105					110																		
TTC	GAT	ATC	ATT	GAC	AAA	GAC	CAA	AAT	GGA	GCT	ATT	TCA	CTG	GAT	GAA	384														
Phe	Asp	Ile	Ile	Asp	Lys	Asp	Gln	Asn	Gly	Ala	Ile	Ser	Leu	Asp	Glu															
	115					120					125																			
TGG	AAA	GCA	TAC	ACC	AAA	TCT	GCT	GGC	ATC	ATC	CAA	TCG	TCA	GAA	GAT	432														
Trp	Lys	Ala	Tyr	Thr	Lys	Ser	Ala	Gly	Ile	Ile	Gln	Ser	Ser	Glu	Asp															
	130				135			140																						
TGC	GAG	GAA	ACA	TTC	AGA	GTG	TGC	GAT	ATT	GAT	GAA	AGT	GGA	CAG	CTC	480														
Cys	Glu	Glu	Thr	Phe	Arg	Val	Cys	Asp	Ile	Asp	Glu	Ser	Gly	Gln	Leu															
	145			150				155						160																
GAT	GTG	GAT	GAG	ATG	ACA	AGA	CAA	CAT	TTA	GGA	TTT	TGG	TAC	ACC	ATG	528														
Asp	Val	Asp	Glu	Met	Thr	Arg	Gln	His	Leu	Gly	Phe	Trp	Tyr	Thr	Met															
			165				170						175																	
GAT	CCT	GCT	TGC	GAA	AAG	CTC	TAC	GGT	GGA	GCT	GTC	CCC				567														
Asp	Pro	Ala	Cys	Glu	Lys	Leu	Tyr	Gly	Gly	Ala	Val	Pro																		
		180				185																								

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 588 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 1...588

(D) OTHER INFORMATION: Aequorin mutant w/increased bioluminescence activity

(x) PUBLICATION INFORMATION:

PATENT NO.: 5,360,728

(K) RELEVANT RESIDUES IN SEQ ID NO: 10:

Asp 124 changed to Ser

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATG ACC AGC GAA CAA TAC TCA GTC AAG CTT ACA CCA GAC TTC GAC AAC	48
Met Thr Ser Glu Gln Tyr Ser Val Lys Leu Thr Pro Asp Phe Asp Asn	
1 5 10 15	
CCA AAA TGG ATT GGA CGA CAC AAG CAC ATG TTT AAT TTT CTT GAT GTC	96
Pro Lys Trp Ile Gly Arg His Lys Met Phe Asn Phe Leu Asp Val	
20 25 30	
AAC CAC AAT GGA AGG ATC TCT CTT GAC GAG ATG GTC TAC AAG GCG TCC	144
Asn His Asn Gly Arg Ile Ser Leu Asp Glu Met Val Tyr Lys Ala Ser	
35 40 45	
GAT ATT GTT ATA AAC AAT CTT GGA GCA ACA CCT GAA CAA GCC AAA CGT	192
Asp Ile Val Ile Asn Asn Leu Gly Ala Thr Pro Glu Gln Ala Lys Arg	
50 55 60	
CAC AAA GAT GCT GTA GAA GCC TTC TTC GGA GGA GCT GCA ATG AAA TAT	240
His Lys Asp Ala Val Glu Ala Phe Phe Gly Gly Ala Ala Met Lys Tyr	
65 70 75 80	
GGT GTA GAA ACT GAA TGG CCT GAA TAC ATC GAA GGA TGG AAA AGA CTG	288
Gly Val Glu Thr Trp Pro Glu Tyr Ile Glu Gly Trp Lys Arg Leu	
85 90 95	
GCT TCC GAG GAA TTG AAA AGG TAT TCA AAA AAC CAA ATC ACA CTT ATT	336
Ala Ser Glu Glu Leu Lys Arg Tyr Ser Lys Asn Gln Ile Thr Leu Ile	
100 105 110	
CGT TTA TGG GGT GAT GCA TTG TTC GAT ATC ATT TCC AAA GAC CAA AAT	384
Arg Leu Trp Gly Asp Ala Leu Phe Asp Ile Ile Ser Lys Asp Gln Asn	
115 120 125	
GGA GCT ATT TCA CTG GAT GAA TGG AAA GCA TAC ACC AAA TCT GCT GGC	432
Gly Ala Ile Ser Leu Asp Glu Trp Lys Ala Tyr Thr Lys Ser Ala Gly	
130 135 140	
ATC ATC CAA TCG TCA GAA GAT TGC GAG GAA ACA TTC AGA GTG TGC GAT	480
Ile Ile Gln Ser Ser Glu Asp Cys Glu Glu Thr Phe Arg Val Cys Asp	
145 150 155 160	
ATT GAT GAA AGT GGA CAG CTC GAT GTT GAT GAG ATG ACA AGA CAA CAT	528
Ile Asp Glu Ser Gly Gln Leu Asp Val Asp Glu Met Thr Arg Gln His	
165 170 175	
TTA GGA TTT TGG TAC ACC ATG GAT CCT GCT TGC GAA AAG CTC TAC GGT	576
Leu Gly Phe Trp Tyr Thr Met Asp Pro Ala Cys Glu Lys Leu Tyr Gly	
180 185 190	
GGA GCT GTC CCC	588
Gly Ala Val Pro	
195	

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 588 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

- (iv) ANTISENSE: NO
 (v) FRAGMENT TYPE:
 (vi) ORIGINAL SOURCE:
 (ix) FEATURE:

(A) NAME/KEY: Coding Sequence
 (B) LOCATION: 1...588
 (D) OTHER INFORMATION: Recombinant site-directed Aequorin mutant
 w/increased biolum. activity

(x) PUBLICATION INFORMATION:

PATENT NO.: 5,360,728

(K) RELEVANT RESIDUES IN SEQ ID NO: 11:
 Glu 135 changed to Ser

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATG ACC AGC GAA CAA TAC TCA GTC AAG CTT ACA CCA GAC TTC GAC AAC	48
Met Thr Ser Glu Gln Tyr Ser Val Lys Leu Thr Pro Asp Phe Asp Asn	
1 5 10 15	
CCA AAA TGG ATT GGA CGA CAC AAG CAC ATG TTT AAT TTT CTT GAT GTC	96
Pro Lys Trp Ile Gly Arg His Lys His Met Phe Asn Phe Leu Asp Val	
20 25 30	
AAC CAC AAT GGA AGG ATC TCT CTT GAC GAG ATG GTC TAC AAG GCG TCC	144
Asn His Asn Gly Arg Ile Ser Leu Asp Glu Met Val Tyr Lys Ala Ser	
35 40 45	
GAT ATT GTT ATA AAC AAT CTT GGA GCA ACA CCT GAA CAA GCC AAA CGT	192
Asp Ile Val Ile Asn Asn Leu Gly Ala Thr Pro Glu Gln Ala Lys Arg	
50 55 60	
CAC AAA GAT GCT GTA GAA GCC TTC TTC GGA GGA GCT GCA ATG AAA TAT	240
His Lys Asp Ala Val Glu Ala Phe Phe Gly Gly Ala Ala Met Lys Tyr	
65 70 75 80	
GGT GTA GAA ACT GAA TGG CCT GAA TAC ATC GAA GGA TGG AAA AGA CTG	288
Gly Val Glu Thr Glu Trp Pro Glu Tyr Ile Glu Gly Trp Lys Arg Leu	
85 90 95	
GCT TCC GAG GAA TTG AAA AGG TAT TCA AAA AAC CAA ATC ACA CTT ATT	336
Ala Ser Glu Glu Leu Lys Arg Tyr Ser Lys Asn Gln Ile Thr Leu Ile	
100 105 110	
CGT TTA TGG GGT GAT GCA TTG TTC GAT ATC ATT TCC AAA GAC CAA AAT	384
Arg Leu Trp Gly Asp Ala Leu Phe Asp Ile Ile Ser Lys Asp Gln Asn	
115 120 125	
GGA GCT ATT TCA CTG GAT TCA TGG AAA GCA TAC ACC AAA TCT GCT GGC	432
Gly Ala Ile Ser Leu Asp Ser Trp Lys Ala Tyr Thr Lys Ser Ala Gly	
130 135 140	
ATC ATC CAA TCG TCA GAA GAT TGC GAG GAA ACA TTC AGA GTG TGC GAT	480
Ile Ile Gln Ser Ser Glu Asp Cys Glu Glu Thr Phe Arg Val Cys Asp	
145 150 155 160	
ATT GAT GAA AGT GGA CAG CTC GAT GTT GAT GAG ATG ACA AGA CAA CAT	528
Ile Asp Glu Ser Gly Gln Leu Asp Val Asp Glu Met Thr Arg Gln His	
165 170 175	
TTA GGA TTT TGG TAC ACC ATG GAT CCT GCT TGC GAA AAG CTC TAC GGT	576
Leu Gly Phe Trp Tyr Thr Met Asp Pro Ala Cys Glu Lys Leu Tyr Gly	

180 185 190 588

GGA GCT GTC CCC
Gly Ala Val Pro
195

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 588 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 1...588

(D) OTHER INFORMATION: Recombinant site-directed
Aequorin mutant w/increased biolum. activity

(x) PUBLICATION INFORMATION:

PATENT NO.: 5,360,728

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ATG ACC AGC GAA CAA TAC TCA GTC AAG CTT ACA CCA GAC TTC GAC AAC	48
Met Thr Ser Glu Gln Tyr Ser Val Lys Leu Thr Pro Asp Phe Asp Asn	
1 5 10 15	
CCA AAA TGG ATT GGA CGA CAC AAG CAC ATG TTT AAT TTT CTT GAT GTC	96
Pro Lys Trp Ile Gly Arg His Lys His Met Phe Asn Phe Leu Asp Val	
20 25 30	
AAC CAC AAT GGA AGG ATC TCT CTT GAC GAG ATG GTC TAC AAG GCG TCC	144
Asn His Asn Gly Arg Ile Ser Leu Asp Glu Met Val Tyr Lys Ala Ser	
35 40 45	
GAT ATT GTT ATA AAC AAT CTT GGA GCA ACA CCT GAA CAA GCC AAA CGT	192
Asp Ile Val Ile Asn Asn Leu Gly Ala Thr Pro Glu Gln Ala Lys Arg	
50 55 60	
CAC AAA GAT GCT GTA GAA GCC TTC TTC GGA GGA GCT GCA ATG AAA TAT	240
His Lys Asp Ala Val Glu Ala Phe Phe Gly Gly Ala Ala Met Lys Tyr	
65 70 75 80	
GGT GTA GAA ACT GAA TGG CCT GAA TAC ATC GAA GGA TGG AAA AGA CTG	288
Gly Val Glu Thr Glu Trp Pro Glu Tyr Ile Glu Gly Trp Lys Arg Leu	
85 90 95	
GCT TCC GAG GAA TTG AAA AGG TAT TCA AAA AAC CAA ATC ACA CTT ATT	336
Ala Ser Glu Glu Leu Lys Arg Tyr Ser Lys Asn Gln Ile Thr Leu Ile	
100 105 110	
CGT TTA TGG GGT GAT GCA TTG TTC GAT ATC ATT TCC AAA GAC CAA AAT	384
Arg Leu Trp Gly Asp Ala Leu Phe Asp Ile Ile Ser Lys Asp Gln Asn	
115 120 125	
GCA GCT ATT TCA CTG GAT GAA TGG AAA GCA TAC ACC AAA TCT GCT GGC	432
Ala Ala Ile Ser Leu Asp Glu Trp Lys Ala Tyr Thr Lys Ser Ala Gly	

130	135	140	
ATC ATC CAA TCG TCA GAA GAT TGC GAG GAA ACA TTC AGA GTG TGC GAT			480
Ile Ile Gln Ser Ser Glu Asp Cys Glu Glu Thr Phe Arg Val Cys Asp			
145	150	155	160
ATT GAT GAA AGT CGA CAG CTC GAT GTT GAT GAG ATG ACA AGA CAA CAT			528
Ile Asp Glu Ser Gly Gln Leu Asp Val Asp Glu Met Thr Arg Gln His			
	165	170	175
TTA GGA TTT TGG TAC ACC ATG GAT CCT GCT TGC GAA AAG CTC TAC GGT			576
Leu Gly Phe Trp Tyr Thr Met Asp Pro Ala Cys Glu Lys Leu Tyr Gly			
	180	185	190
GGA GCT GTC CCC			588
Gly Ala Val Pro			
195			

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 567 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: Coding Sequence

(B) LOCATION: 1...567

(D) OTHER INFORMATION: Recombinant apoaeguorin (AQUALITE®)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GTC AAG CTT ACA CCA GAC TTC GAC AAC CCA AAA TGG ATT GGA CGA CAC		48
Val Lys Leu Thr Pro Asp Phe Asp Asn Pro Lys Trp Ile Gly Arg His		
1	5	10
AAG CAC ATG TTT AAT TTT CTT GAT GTC AAC CAC AAT GGA AGG ATC TCT		96
Lys His Met Phe Asn Phe Leu Asp Val Asn His Asn Gly Arg Ile Ser		
	20	25
CTT GAC GAG ATG GTC TAC AAG GCG TCC GAT ATT GTT ATA AAC AAT CTT		144
Leu Asp Glu Met Val Tyr Lys Ala Ser Asp Ile Val Ile Asn Asn Leu		
	35	40
GGA GCA ACA CCT GAA CAA GCC AAA CGT CAC AAA GAT GCT GTA GAA GCC		192
Gly Ala Thr Pro Glu Gln Ala Lys Arg His Lys Asp Ala Val Glu Ala		
	50	55
TTC TTC GGA GGA GCT GGA ATG AAA TAT GGT GTA GAA ACT GAA TGG CCT		240
Phe Phe Gly Gly Ala Gly Met Lys Tyr Gly Val Glu Thr Glu Trp Pro		
	65	70
GAA TAC ATC GAA GGA TGG AAA AAA CTG GCT TCC GAG GAA TTG AAA AGG		288
Glu Tyr Ile Glu Gly Trp Lys Lys Leu Ala Ser Glu Glu Leu Lys Arg		
	85	90
TAT TCA AAA AAC CAA ATC ACA CTT ATT CGT TTA TGG GGT GAT GCA TTG		336
Tyr Ser Lys Asn Gln Ile Thr Leu Ile Arg Leu Trp Gly Asp Ala Leu		
	100	105
TTC GAT ATC ATT GAC AAA GAC CAA AAT GGA GCT ATT CTG TCA GAT GAA		384

Phe	Asp	Ile	Ile	Asp	Lys	Asp	Gln	Asn	Gly	Ala	Ile	Leu	Ser	Asp	Glu		
	115						120					125					
TGG	AAA	GCA	TAC	ACC	AAA	TCT	GAT	GGC	ATC	ATC	CAA	TCG	TCA	GAA	GAT	432	
Trp	Lys	Ala	Tyr	Thr	Lys	Ser	Asp	Gly	Ile	Ile	Gln	Ser	Ser	Glu	Asp		
	130					135				140							
TGC	GAG	GAA	ACA	TTC	AGA	GTG	TGC	GAT	ATT	GAT	GAA	AGT	GGA	CAG	CTC	480	
Cys	Glu	Glu	Thr	Phe	Arg	Val	Cys	Asp	Ile	Asp	Glu	Ser	Gly	Gln	Leu		
	145				150				155					160			
GAT	GTT	GAT	GAG	ATG	ACA	AGA	CAA	CAT	TTA	GGA	TTT	TGG	TAC	ACC	ATG	528	
Asp	Val	Asp	Glu	Met	Thr	Arg	Gln	His	Leu	Gly	Phe	Trp	Tyr	Thr	Met		
				165				170						175			
GAT	CCT	GCT	TGC	GAA	AAG	CTC	TAC	GGT	GGA	GCT	GTC	CCC				567	
Asp	Pro	Ala	Cys	Glu	Lys	Leu	Tyr	Gly	Gly	Ala	Val	Pro					
				180				185									

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 236 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(x) PUBLICATION INFORMATION:

PATENT NO.: 5,484,723

(ix) FEATURE:

- (D) OTHER INFORMATION: Vibrio fisheri Flavín reductase

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met	Pro	Ile	Asn	Cys	Lys	Val	Lys	Ser	Ile	Glu	Pro	Leu	Ala	Cys	Asn		
1				5					10					15			
Thr	Phe	Arg	Ile	Leu	Leu	His	Pro	Glu	Gln	Pro	Val	Ala	Phe	Lys	Ala		
			20					25					30				
Gly	Gln	Tyr	Leu	Thr	Val	Val	Met	Gly	Glu	Lys	Asp	Lys	Arg	Pro	Phe		
		35					40					45					
Ser	Ile	Ala	Ser	Ser	Pro	Cys	Arg	His	Glu	Gly	Glu	Ile	Glu	Leu	His		
	50					55					60						
Ile	Gly	Ala	Ala	Glu	His	Asn	Ala	Tyr	Ala	Gly	Glu	Val	Val	Glu	Ser		
	65				70					75				80			
Met	Lys	Ser	Ala	Leu	Glu	Thr	Gly	Gly	Asp	Ile	Leu	Ile	Asp	Ala	Pro		
			85					90						95			
His	Gly	Glu	Ala	Trp	Ile	Arg	Glu	Asp	Ser	Asp	Arg	Ser	Met	Leu	Leu		
			100					105					110				
Ile	Ala	Gly	Gly	Thr	Gly	Phe	Ser	Tyr	Val	Arg	Ser	Ile	Leu	Asp	His		
		115						120					125				
Cys	Ile	Ser	Gln	Gln	Ile	Gln	Lys	Pro	Ile	Tyr	Leu	Tyr	Trp	Gly	Gly		
	130					135					140						
Arg	Asp	Glu	Cys	Gln	Leu	Tyr	Ala	Lys	Ala	Glu	Leu	Glu	Ser	Ile	Ala		

145		150		155		160
Gln Ala His Ser	His Ile Thr Phe Val	Pro Val Val Glu Lys Ser Glu				
	165	170				175
Gly Trp Thr Gly	Lys Thr Gly Asn Val Leu Glu Ala Val Lys Ala Asp					
	180	185				190
Phe Asn Ser Leu Ala Asp Met Asp	Ile Tyr Ile Ala Gly Arg Phe Glu					
	195	200				205
Met Ala Gly Ala Ala Arg Glu Gln Phe Thr Thr Glu Lys Gln Ala Lys						
	210	215				220
Lys Glu Gln Leu Phe Gly Asp Ala Phe Ala Phe Ile						
225	230			235		